CONTROLLED RELEASE THERAPEUTIC WOUND DRESSINGS

The present invention relates to wound dressing materials, and in particular to new materials for the controlled release of therapeutic agents into wounds.

All publications, patents and patent applications cited herein are incorporated in full by reference.

In mammals, injury triggers an organised complex cascade of cellular and biochemical events that result in a healed wound. Wound healing is a complex dynamic process that results in the restoration of anatomic continuity and function; an ideally healed wound is one that has returned to normal anatomic structure, function and appearance.

Infection of wounds by bacteria delays the healing process, since bacteria compete for nutrients and oxygen with macrophages and fibroblasts, whose activities are essential for the healing of the wound. Infection results when bacteria achieve dominance over the systemic and local factors of host resistance. Infection is therefore a manifestation of a disturbed host/bacteria equilibrium in favour of the invading bacteria. This elicits a systemic septic response, and also inhibits the multiple processes involved in wound healing. Lastly, infection can result in a prolonged inflammatory phase and thus slow healing, or may cause further necrosis of the wound. The granulation phase of the healing process will begin only after the infection has subsided.

Chronically contaminated wounds all contain tissue bacterial flora. These bacteria may be indigenous to the patient or might be exogenous to the wound. Closure, or eventual healing of the wound is often based on a physician's ability to control the level of the bacterial flora.

If clinicians could respond to wound infection as early as possible the infection could be treated topically as opposed to having to use antibiotics. This would also

lead to less clinical intervention/hospitalisation and would reduce the use of antibiotics and other complications of infection.

Current methods used to identify bacterial infection rely mainly on judgement of the odour and appearance of a wound. With experience, it is possible to identify an infection in a wound by certain chemical signs such as redness or pain. Some clinicians take swabs that are then cultured in the laboratory to identify specific organisms, but this technique takes time.

It has now been discovered that wound fluid from wounds that are apparently not clinically infected but which go on to become infected within a few days have increased levels of neutrophil elastase activity.

Further, chronic wounds, such as venous ulcers, pressure sores and diabetic ulcers have a disordered wound healing metabolism even in the absence of infection. In particular, wound chronicity is associated with elevated levels of protease enzymes in the wound that interfere with the normal processes of tissue formation and destruction in the wound.

It is known to provide antimicrobial wound dressings. For example, such dressings are known having a liquid permeable wound contacting layer, an intermediate absorbent layer and an outer, liquid-impervious backing layer, in which one or more of the layers contains an antimicrobial agent. For example, EP-A-0599589 describes layered wound dressings having a wound contacting layer of a macromolecular hydrocolloid, an absorbent layer, and a continuous, microporous sheet intermediate the wound contacting layer and the absorbent layer. The absorbent layer contains a low molecular weight antimicrobial agent that can diffuse into the wound.

WO-A-238097 describes wound dressings comprising a liquid-permeable top sheet having a wound facing surface and a back surface, and a hydrogel layer on the wound facing surface of the top sheet. The top sheet is adapted to block or restrict passage of liquid from the back surface to the wound facing surface. The

hydrogel layer is an insoluble hydrogel adapted to maintain a moist wound healing environment at the wound surface. The hydrogel may contain therapeutic agents, such as antimicrobial agents, for sustained release into the wound.

Previous antimicrobial wound dressings suffer from the drawback that the release of the antimicrobial agent is relatively unresponsive to the degree of infection of the wound being treated. This is undesirable because it can result in resistant microorganisms, and also because all unnecessary medication can interfere with the processes of wound healing.

Copending UK patent application no. 0129292.9 filed 6th December 2001 (the content of which is incorporated herein by reference) discloses a bioresorbable barrier composed of a range of natural materials including elastin. Sheets composed of elastin would therefore be expected to be degraded in the presence of elevated levels of elastase that would be expected to occur during infection.

However, it is thought that metalloproteinases (MMPs) have an ability to degrade elastin and certain MMPs (e.g. the 72kDa and 92kDa gelatinases) are elevated in chronic, non-infected wounds. MMPs include matrix gelatinases, stromelysins and collagenases. Of the known MMPs, the only enzymes currently known to degrade insoluble elastin (apart from elastases) are the 92- and 72-kDa (MMP2) gelatinases, MMP9 and matrilysin (Mecham, R.P., *et al.* (1997) J. Biol. Chem. 272(29), 18071-6 and Senior, R.M., et al (1991) J. Biol. Chem. 266, 7870-5.). It is therefore likely that elastin sheets would be degraded in the absence of elevated levels of elastase by proteases elevated in normal non-infected chronic wounds.

With regard to cleavage sites of elastase and MMPs the reader is referred to Mecham *et al.* As discussed in Mecham *et al.* elastin is degraded by neutrophil elastase at sequences Gly/Ala/Val-Gly or Gly/Ala-Ala. Neutrophil elastase, in contrast to MMPs, is a serine protease and prefers non-bulky amino acids in its degradation sequences, and has a Gly or Ala in its P1 position (see Mecham *et al.* for discussion of the P1 position). In contrast, the sequences cleaved by MMP's are characteristically adjacent to hydrophilic aliphatic residues such as Leu and Ile

in elastin and MMPs typically have Ile or Leu in its P1 position. In addition, it has also been shown that in elastin Pro-Xaa-(Gly/Ala)-Leu/Ile or pro-Xaa-Ala-Ala-Leu/Ile (Xaa indicates that any amino acid may be present) are degraded by gelatinase (see Mecham *et al.*), which are again distinct from those degraded by elastase. Similarly, Pro-Xaa-(Gly/Ala)-Val/Phe and pro-Xaa-Ala-Ala-Val/Phe are also degraded by gelatinase, although these sequences are not as susceptible to cleavage as Pro-Xaa-(Gly/Ala)-Leu/Ile or pro-Xaa-Ala-Ala-Leu/Ile.

The present invention provides wound dressings which comprise modified elastin (including derivatives of elastin) that have been modified in such a way that they are less susceptible (and preferably no longer susceptible) to degradation by non-elastase proteases that may be found in the wound environment, and particularly in non-infected wound environments, such as metalloproteinases.

It is proposed to produce modified recombinant elastin with the Pro-Xaa-(Gly/Ala)-Leu/Ile, Pro-Xaa-Ala-Ala-Leu/Ile, Pro-Xaa-(Gly/Ala)-Val/Phe or Pro-Xaa-Ala-Ala-Val/Phe sequences modified in such a way that they are less susceptible (and preferably no longer susceptible) to degradation by a non-elastase protease. Thus, elastin which is less susceptible (and which is preferably no longer susceptible) to degradation by non-elastase proteases is provided by the present invention.

In addition, it is possible that by using recombinant methods elastin could be produced with additional elastase-sensitive regions which are selectively degraded by neutrophil elastase. In this way elastin films or sheets can be designed to degrade with defined kinetics following elevation of elastase levels.

Accordingly, a first aspect of the invention comprises a wound dressing comprising a therapeutic agent and a barrier layer for initially separating the therapeutic agent from a wound fluid when in use, wherein the barrier layer comprises, and preferably consists of, modified elastin wherein

(i) the elastin has been modified so that it is less susceptible to degradation by one or more non-elastase protease found in wound fluid; or

(ii) the elastin has been modified so that there are additional elastase sensitive regions which are selectively degraded by elastase.

Preferably, the non-elastase protease is a matrix metalloproteinase (e.g. preferably the 72kDa gelatinase (MMP2), the 92kDa gelatinase (MMP9), matrilysin or metalloelastase) or a serine protease.

Unless otherwise indicated, by "elastase" we include neutrophil elastase and bacterial elastases present in infected wounds. Preferably, the elastase is human neutrophil elastase.

Preferably, the elastin has been modified so that it is less susceptible to degradation by one or more non-elastase proteases and has been modified so that there are additional elastase sensitive regions which are selectively degraded by elastase.

Elastin is comprised of cross-linked tropoelastin monomers. The recombinant production of tropoelastins is well known in the art. See, for example, US 6,232,458, WO 99/03886 and WO 94/14958. The term "elastin" as used herein includes (but is not limited to) the elastin formed by the cross-linking of tropoelastin molecules obtainable by the methods described in US 6,232,458, WO 99/03886 and WO 94/14958. Various methods of cross-linking tropoelastin monomers are known in the art and include those methods described in US 6,232,458, WO 99/03886 and WO 94/14958, the contents of which are incorporated herein by reference.

For the avoidance of doubt, the term tropoelastin is intended to include tropoelastin "derivatives", "variants" etc. as described in US 6,232,458, WO 99/03886 and WO 94/14958 whilst the term "elastin" is intended to include elastin, "elastin-like material", "elastin-like products" etc. as described in US 6,232,458, WO 99/03886 and WO 94/14958. Thus, the term "elastin" as used

herein need not necessarily refer to 'full length' elastin but may include peptide sequences based on smaller portions of elastin.

By "modified elastin" we refer to elastin which has been modified so as to decrease its susceptiblity to cleavage by one or more non-elastase proteases (the non-elastase proteases may be acting singly or in combination – as noted below non-elastase proteases may act synergistically to degrade elastin) which may be found in the wound environment, and particularly in non-infected wound environments, or to elastin which has been modified to increase its susceptiblity to elastase by the provision of additional elastase sensitive regions which are selectively degraded by elastase. However, the modifications to the elastin should not be such that the properties of the resulting structure no longer resemble wild-type elastin.

When modifying sequences in the elastin to render the elastin less susceptible to cleavage by one or more non-elastase proteases, the modified sequences should ideally be chosen to avoid the creation of sequences which may be cleaved by another non-elastase protease (e.g. collagenases) found in the wound environment and, in particular, in non-infected wound environments. Various prior art references disclose sequences cleaved by non-elastase proteases, see for example WO 00/64486. Further, Mecham et al. describes methods for identifying cleavage sites in addition to disclosing cleavage sites of various MMPs. Thus, when modifying sequences in the elastin which are susceptible to cleavage by non-elastase proteases found in the wound environment, it is preferred that the sequences are modified in such a way that they are not susceptible to cleavage by any other non-elastase protease found in the wound environment.

In order to decrease elastin's susceptiblity to non-elastase proteases, the elastin may be modified so that at least some of the Pro-Xaa-(Gly/Ala)-Leu/Ile, Pro-Xaa-Ala-Ala-Leu/Ile, Pro-Xaa-(Gly/Ala)-Val/Phe or Pro-Xaa-Ala-Ala-Val/Phe sequences are modified so that the sequences are less susceptible, and preferably no longer susceptible, to cleavage by a non-elastase protease, such as the 72 or 92kDa gelatinases.

For instance, changing the Leu/Ile in Pro-Xaa-(Gly/Ala)-Leu/Ile or Pro-Xaa-Ala-Ala-Leu/Ile sequence to Val or Phe would make the sequence less susceptible to MMP attack. Changing the Leu/Ile to an amino acid other than Val or Phe (e.g. to His, Glu, Asp, Lys etc) should almost eliminate its ability to be cleaved in the presence of non-elastase proteases. Changing the Leu/Ile in Pro-Xaa-(Gly/Ala)-Leu/Ile or Pro-Xaa-Ala-Ala-Leu/Ile to Gly or Ala may make these sequences susceptible to elastase if the amino terminal amino acid corresponds to Val, Gly, or Ala. Changing to other amino acids (e.g. lysine, arginine, serine glutamine etc.) would be expected to render the sequence not degradable by elastase or a MMP.

Alternatively, changing the Proline in Pro-Xaa-(Gly/Ala)-Leu/Ile, Pro-Xaa-Ala-Ala-Leu/Ile, Pro-Xaa-(Gly/Ala)-Val/Phe or Pro-Xaa-Ala-Ala-Val/Phe is another means of reducing the ability to be degraded by MMPs. Preferably, the proline is substituted with phenylalanine. However, as Proline residues are known to have an important effect on protein architecture caution should be exercised not to change too many prolines (particularly to non- phenylalanine residues) as this could change the properties of the elastin to such an extent that the structure and its properties no longer resemble wild-type elastin.

Those skilled in the art will be readily able to devise methods for modifying elastin in order to render it less susceptible to attack by one or more non-elastase protease. The sequences can be modified using standard molecular biological recombinant DNA techniques. The sequences may, for example, be modified by amino acid deletions, insertions or substitutions. The substitutions, deletions or insertions may involve one or more amino acids. Techniques for the genetic manipulation of sequences will be well known to those skilled in the art and such techniques are explained fully in the literature. See, for example, Sambrook *et al.* Molecular Cloning; A Laboratory Manual, Third Edition (2001).

To verify that a modification results in a reduction in susceptibility to cleavage by one or more non-elastase proteases, electrophoresis could be used to ensure that a reduced number of small molecular weight peptides (and preferably no small

molecular weight peptides) are produced by degradation when the non-elastase protease (or non-elastase proteases) is mixed with the new recombinant elastin. Since non-elastase proteases may act synergistically to degrade elastin, and since various non-elastase proteases will be present in the wound environment, it is preferred that the effect of a modification is assessed by subjecting the modified sequence to more than one non-elastase protease (e.g. 3, 4, or 5 non-elastase proteases).

Preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, of the Pro-Xaa-Gly-Leu sequences have been modified in such a way that they are less susceptible, and preferably no longer susceptible, to cleavage by the 92k Da gelatinase.

Preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, of the Pro-Xaa-Gly-Ile sequences have been modified in such a way that they are less susceptible, and preferably no longer susceptible, to cleavage by the 92k Da gelatinase.

Preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, of the Pro-Xaa-Ala-Leu sequences have been modified in such a way that they are less susceptible, and preferably no longer susceptible, to cleavage by the 92k Da gelatinase.

Preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, of the Pro-Xaa-Ala-Ile sequences have been modified in such a way that they are less susceptible, and preferably no longer susceptible, to cleavage by the 92k Da gelatinase.

Preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, of the Pro-Xaa-Ala-Ala-Leu sequences have been modified in such a way that they are less susceptible (e.g. to Pro-Xaa-Glu-Val-Leu), and preferably no longer susceptible (e.g. to Pro-Xaa-Glu-Val- Val), to cleavage by the 92k Da gelatinase.

Preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, of the Pro-Xaa-Ala-Ala-Ile sequences have been modified in such a way that they are less susceptible, and preferably no longer susceptible, to cleavage by the 92k Da gelatinase.

Preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, of the Pro-Xaa-Gly-Val sequences have been modified in such a way that they are less susceptible, and preferably no longer susceptible, to cleavage by the 92k Da gelatinase.

Preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, of the Pro-Xaa-Gly-Phe sequences have been modified in such a way that they are less susceptible, and preferably no longer susceptible, to cleavage by the 92k Da gelatinase.

Preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, of the Pro-Xaa-Ala-Val sequences have been modified in such a way that they are less susceptible, and preferably no longer susceptible, to cleavage by the 92k Da gelatinase.

Preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, of the Pro-Xaa-Ala-Phe sequences have been modified in such a way that they are less susceptible, and preferably no longer susceptible, to cleavage by the 92k Da gelatinase.

Preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, of the Pro-Xaa-Ala-Ala-Val sequences have been modified in such a way that they are less susceptible, and preferably no longer susceptible, to cleavage by the 92k Da gelatinase.

Preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, of the Pro-Xaa-Ala-Ala-Phe sequences have been modified in such a way that

they are less susceptible, and preferably no longer susceptible, to cleavage by the 92k Da gelatinase.

Preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, of the total number of the Pro-Xaa-Gly-Leu, Pro-Xaa-Gly-Ile, Pro-Xaa-Ala-Leu, Pro-Xaa-Ala-Ile, Pro-Xaa-Ala-Ala-Leu, Pro-Xaa-Ala-Ala-Ile, Pro-Xaa-Gly-Val, Pro-Xaa-Gly-Phe, Pro-Xaa-Ala-Val, Pro-Xaa-Ala-Phe, Pro-Xaa-Ala-Ala-Val, and Pro-Xaa-Ala-Ala-Phe sequences have been modified in such a way that they are less susceptible, and preferably no longer susceptible, to cleavage by the 92k Da gelatinase.

Preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, of each of the Pro-Xaa-Gly-Leu, Pro-Xaa-Gly-Ile, Pro-Xaa-Ala-Leu, Pro-Xaa-Ala-Ile, Pro-Xaa-Ala-Ala-Leu, Pro-Xaa-Ala-Ala-Ile, Pro-Xaa-Gly-Val, Pro-Xaa-Gly-Phe, Pro-Xaa-Ala-Val, Pro-Xaa-Ala-Phe, Pro-Xaa-Ala-Ala-Val, and Pro-Xaa-Ala-Ala-Phe sequences have been modified in such a way that they are less susceptible, and preferably no longer susceptible, to cleavage by the 92k Da gelatinase.

In an alternative or additional embodiment of the first aspect of the invention, the elastin has been modified so that there are additional elastase-sensitive regions which are selectively degraded by elastase. In one embodiment, the elastin contains additional Gly/Ala/Val-Gly or Gly/Ala-Ala sequences.

By elastase-sensitive regions which are "selectively degraded by elastase" we refer to sequences which are cleavable by elastase but which are not cleavable by non-elastase proteases that may be found in the wound environment, and particularly in non-infected wound environments. Thus, elastase-sensitive regions which are "selectively degraded by elastase" are not cleavable by proteases such as gelatinases, collagenases and metalloproteinases.

As will be appreciated from the foregoing, the additional Gly/Ala/Val-Gly or Gly/Ala-Ala sequences should be chosen with regard to those sequences which are degraded by non-elastase proteases (eg MMPs) found in the wound

environment, to ensure that the additional Gly/Ala/Val-Gly or Gly/Ala-Ala sequences are not also degraded by a non-elastase protease such as a MMP2 or MMP9. Thus, for instance additional Pro-Ala-Gly-Leu sequences would not qualify as additional elastase-sensitive regions which are selectively degraded by elastase since such sequences are susceptible to degradation by a MMP. However, as indicated above if Pro-Xaa-Gly-Ile was modified by replacement of Ile with Gly this would not only result in a sequence with reduced susceptibility to cleavage by a non-elastase protease but would also result in a sequence which is susceptible to elastase cleavage.

In a preferred embodiment a sequence susceptible to cleavage by a non-elastase protease (e.g. Pro-Xaa-Gly-Leu, Pro-Xaa-Gly-Ile, Pro-Xaa-Ala-Leu, Pro-Xaa-Ala-Ile, Pro-Xaa-Ala-Ala-Leu, Pro-Xaa-Ala-Ala-Ile, Pro-Xaa-Gly-Val, Pro-Xaa-Gly-Phe, Pro-Xaa-Ala-Val, Pro-Xaa-Ala-Phe, Pro-Xaa-Ala-Ala-Val, or Pro-Xaa-Ala-Ala-Phe) is modified to render it less susceptible to cleavage by a non-elastase protease but more susceptible to cleavage by elastase.

As mentioned above, techniques for the genetic manipulation of sequences are well known to those skilled in the art and such techniques are explained fully in the literature. See, for example, Sambrook *et al.* Molecular Cloning; A Laboratory Manual, Third Edition (2001).

Preferably, the elastin has been modified so as to increase the number of elastase-sensitive regions which are selectively degraded by elastase by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200% or 250%.

Preferably, the elastin has been modified so as to increase the number of Ala-Gly sequences by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200% or 250%.

Preferably, the elastin has been modified so as to increase the number of Val-Gly sequences by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200% or 250%.

Preferably, the elastin has been modified so as to increase the number of Ala-Ala sequences by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200% or 250%.

Preferably, the elastin has been modified so as to increase the number of Gly-Ala sequences by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200% or 250%.

The therapeutic agent may be selected from the group consisting of antimicrobial substances, pain relieving substances, protease inhibitors, and mixtures thereof.

In one embodiment of the first aspect of the invention, the wound dressing comprises an antimicrobial substance. The antimicrobial agent may, for example, comprise an antiseptic, an antibiotic, or mixtures thereof. Preferred antibiotics include tetracycline, penicillins, terramycins, erythromycin, bacitracin, neomycin, polymycin B, mupirocin, clindamycin and mixtures thereof. Preferred antiseptics include silver sulfadiazine, chlorhexidine, povidone iodine, triclosan, other silver salts, sucralfate, quaternary ammonium salts and mixtures thereof. The pain relieving agent may be an analgesic or a local anaesthetic.

The barrier layer is separate from the therapeutic agent, and the therapeutic agent is initially prevented from contacting the wound fluid by the barrier layer. That is to say, the bioavailability of the therapeutic agent to the wound surface is low until the barrier material has been broken down by elastase, at which point the bioavailability increases sharply. Since elastase levels are elevated in infected wounds, this provides for accelerated or selective release of the therapeutic agent into such wounds. The barrier layer is normally substantially impervious to wound fluid and insoluble therein unless the wound fluid contains a sufficient level of elastase enzyme to break down the substrate material.

The barrier layer is preferably about 0.1 to about 3 mm thick. Preferably about 0.5 to 1.5 mm thick. The modified elastin material may be combined in a film-forming

composition with additional polymeric materials, plasticisers, and humectants. Suitable polymers include alginates, guar gum, carboxymethyl cellulose, methyl cellulose, hydroxypropyl methyl cellulose, locust bean gum, carrageenan, chitosan, heparan sulfate, dermatan sulfate, glycosaminoglycans such as hyaluronic acid, proteoglycans, and mixtures thereof. Suitable plasticisers include C2-C8 polyhydric alcohols such as glycerol. Preferably modified elastin material makes up at least about 10%, 15%, 20%, 25%, 30% or 35% by weight of the film-forming composition.

In certain embodiments the barrier layer comprises a substantially continuous film comprising the film forming composition of the modified elastin material as described above.

In other embodiments the barrier layer comprises an apertured sheet having a composition comprising the substrate material applied thereto in occlusive fashion. The occlusive composition may be similar to the film-forming composition described above. In these embodiments, the apertures typically make up from about 0.1% to about 50% of the area of the wound facing surface of the sheet before swelling, more typically from about 1% to about 30% of the area of the apertured sheet, and preferably from about 10% to about 25% of the area of the apertured sheet. Typically, the apertured sheet has from about 1 to about 30 apertures per square cm, for example from about 4 to about 15 apertures per square cm or from about 5 to about 10 apertures per square cm. In certain embodiments the apertures are uniformly distributed over the surface of the sheet, preferably in a regular pattern. The mean area of each aperture may for example be from about 0.01 to about 10 mm^2, preferably from about 0.1 to about 4 mm^2, and more preferably from about 1mm^2 to about 2mm^2. It will be appreciated that the sheet may include more than one size and shape of aperture in order to provide apertures that open more or less quickly on exposure to infected wound fluid. This enables still more control over the dynamics of therapeutic agent delivery to the wound. Typically, substantially the whole area of the apertures in the apertured sheet is blocked by the barrier material before exposure to wound exudate

Preferably, the thickness of the barrier film or the apertured sheet (by ASTM D374-79) is from about 0.2 to about 5 mm, more preferably from about 0.4 to about 3 mm.

For example, the barrier layer material may further comprise a polymer selected from the group consisting of water soluble macromolecular materials (hydrogels) such as sodium alginate, sodium hyaluronate, alginate derivatives such as the propylene glycol alginate described in EP-A-0613692, and soluble hydropolymers formed from vinyl alcohols, vinyl esters, vinyl ethers and carboxy vinyl monomers, meth(acrylic) acid, acrylamide, N-vinyl pyrrolidone, acylamidopropane sulphonic acid, PLURONIC (Registered Trade Mark) (block polyethylene glycol, block polypropylene glycol) polystyrene-, maleic acid, NN-dimethylacrylamide diacetone acrylamide, acryloyl morpholine, and mixtures thereof. Suitable hydrogels are also described in US-A-5352508.

The barrier layer material may further comprise a polymer selected from the group consisting of bioerodible polymers such as polylactide/polyglycolide, collagen, gelatin, polyacrylate gels such as those described in EP-A-0676457, calcium alginate gels, cross-linked hyaluronate gels, gels of alginate derivatives such as propylene glycol alginate, and gels wherein the hydropolymer is formed from vinyl alcohols, vinyl esters, vinyl ethers and carboxy vinyl monomers, meth(acrylic) acid, acrylamide, N-vinyl pyrrolidone, acylamidopropane sulphonic acid, PLURONIC (Registered Trade Mark) (block polyethylene glycol, block polypropylene glycol) polystyrene-, maleic acid, NN-dimethylacrylamide diacetone acrylamide, acryloyl morpholine, and mixtures thereof. Suitable hydrogels are also described in US-A-5352508.

The barrier layer material may further comprise from about 5 to about 50% by weight, preferably from 15 to 40% by weight, on the same basis of one or more humectants such as glycerol. The barrier layer material may further contain up to about 30% w/w, more preferably up to about 15% w/w on the same basis of water.

In certain embodiments wound dressings have a layered structure wherein preferably a layer of the antimicrobial substance is provided behind the barrier layer. That is to say, on the side of the barrier layer opposite to the wound facing surface of the barrier layer in use. The layer of antimicrobial substance may contact the barrier layer directly, or may be separated therefrom for example by an absorbent layer.

Preferably, the barrier sheet according to these embodiments of the invention forms part of a layered wound dressing having the antimicrobial material disposed on the side of the barrier sheet opposite to the wound facing side of the barrier sheet.

Preferably, the layered wound dressing further comprises an absorbent layer or a backing layer.

The area of the optional absorbent layer is typically in the range of from 1cm^2 to 200cm^2, more preferably from 4cm^2 to 100cm^2.

The optional absorbent layer may be any of the layers conventionally used for absorbing wound fluids, serum or blood in the wound healing art, including gauzes, nonwoven fabrics, superabsorbents, hydrogels and mixtures thereof. Preferably, the absorbent layer comprises a layer of absorbent foam, such as an open celled hydrophilic polyurethane foam prepared in accordance with EP-A-0541391, the entire content of which is expressly incorporated herein by reference. In other embodiments, the absorbent layer may be a nonwoven fibrous web, for example a carded web of viscose staple fibers. The basis weight of the absorbent layer may be in the range of 50-500g/m^2, such as 100-400g/m^2. The uncompressed thickness of the absorbent layer may be in the range of from 0.5mm to 10mm, such as 1mm to 4mm. The free (uncompressed) liquid absorbency measured for physiological saline may be in the range of 5 to 30 g/g at 25°. In certain embodiments the antimicrobial material may be dispersed in or on the absorbent layer.

Preferably, the dressing further comprises a backing layer covering the barrier sheet and the optional absorbent layer on the side opposite the wound-facing side of the dressing. The backing layer preferably provides a barrier to passage of microorganisms through the dressing and further preferably blocks the escape of wound fluid from the dressing. The backing layer may extend beyond at least one edge of the barrier sheet and optional absorbent layer to provide an adhesive-coated margin adjacent to the said edge for adhering the dressing to a surface, such as to the skin of a patient adjacent to the wound being treated. An adhesive-coated margin may extend around all sides of the barrier sheet and optional absorbent layer, so that the dressing is a so-called island dressing. However, it is not necessary for there to be any adhesive-coated margin.

Preferably, the backing layer is substantially liquid-impermeable. The backing sheet is preferably semipermeable. That is to say, the backing sheet is preferably permeable to water vapour, but not permeable to liquid water or wound exudate. Preferably, the backing sheet is also microorganism-impermeable. Suitable continuous conformable backing sheets will preferably have a moisture vapor transmission rate (MVTR) of the backing sheet alone of 300 to 5000 g/m^2/24hrs, preferably 500 to 2000 g/m^2/24hrs at 37.5 °C at 100% to 10% relative humidity difference. The backing sheet thickness is preferably in the range of 10 to 1000 micrometers, more preferably 100 to 500 micrometers.

Suitable polymers for forming the backing sheet include polyurethanes and poly alkoxyalkyl acrylates and methacrylates such as those disclosed in GB-A-1280631. Preferably, the backing sheet comprises a continuous layer of a high density blocked polyurethane foam that is predominantly closed-cell. A suitable backing sheet material is the polyurethane film available under the Registered Trade Mark ESTANE 5714F.

The adhesive layer (where present) should be moisture vapor transmitting or patterned to allow passage of water vapor therethrough. The adhesive layer is preferably a continuous moisture vapor transmitting, pressure-sensitive adhesive layer of the type conventionally used for island-type wound dressings, for example,

a pressure sensitive adhesive based on acrylate ester copolymers, polyvinyl ethyl ether and polyurethane as described for example in GB-A-1280631. The basis weight of the adhesive layer is preferably 20 to 250 g/m^2, and more preferably 50 to 150 g/m^2. Polyurethane-based pressure sensitive adhesives are preferred.

Preferably, the adhesive layer extends outwardly from the absorbent layer and the envelope to form an adhesive-coated margin on the backing sheet around the absorbent layer as in a conventional island dressing.

Also within the scope of the present invention are embodiments in which the barrier layer substantially encapsulates the antimicrobial substance. For example, the dressing may comprise, or consist essentially of, particles such as microspheres of antimicrobial material encapsulated in a layer comprising the substrate material. The particles are preferably loaded with from 1 to 90 wt.%, more preferably from 3 to 50 wt.% of the antimicrobial agents.

The particles may be made by any suitable technique, including comminution, coacervation, or two-phase systems for example as described in US-A-3886084. Techniques for the preparation of medicated microspheres for drug delivery are reviewed, for example, in Polymeric Nanoparticles and Microspheres, Guiot and Couvreur eds., CRC Press (1986).

A preferred method for preparation of the microparticles is coacervation, which is especially suited to the formation of particles in the preferred size range of 100 to 500 micrometers having a high loading of therapeutic agents. Coacervation is the term applied to the ability of a number of aqueous solutions of colloids, to separate into two liquid layers, one rich in colloid solute and the other poor in colloid solute. Factors which influence this liquid-liquid phase separation are: (a) the colloid concentration, (b) the solvent of the system, (c) the temperature, (d) the addition of another polyelectrolyte, and (e) the addition of a simple electrolyte to the solution. Coacervation can be of two general types. The first is called "simple" or "salt" coacervation where liquid phase separation occurs by the addition of a simple electrolyte to a colloidal solution. The second is termed "complex" coacervation

where phase separation occurs by the addition of a second colloidal species to a first colloidal solution, the particles of the two dispersed colloids being oppositely charged. Generally, materials capable of exhibiting an electric charge in solution (i.e. materials which possess an ionizable group) are coacervable. Such materials include natural and synthetic macromolecular species such as gelatin, acacia, tragacanth, styrene-maleic anhydride copolymers, methyl vinyl ether-maleic anhydride copolymers, polymethacrylic acid, and the like.

If, prior to the initiation of coacervation, a water-immiscible material, such as an oil, is dispersed as minute droplets in an aqueous solution or sol or an encapsulating colloidal material, and then, a simple electrolyte, such as sodium sulfate, or another, oppositely charged colloidal species is added to induce coacervation, the encapsulating colloidal material forms around each oil droplet, thus investing each of said droplets in a liquid coating of the coacervated colloid. The liquid coatings which surround the oil droplets must thereafter be hardened by cross-linking to produce solid-walled microcapsules

Preferably, the wound dressing according to any aspect of the present invention is sterile and packaged in a microorganism-impermeable container.

A second aspect of the invention provides modified elastin as described in the first aspect of the invention.

A third aspect of the invention provides a polynucleotide sequence encoding a modified tropoelastin polypeptide which polypeptide may be cross-linked with other such tropoelastin polypeptides to form the modified elastin of the second aspect of the invention.